European Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 90 11 8953

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
X	WO-A-8 800 970 (REGENTS OF THE UNIVERSITY OF MINNESOTA) * page 3, line 31 - page 4, line 14 * * page 10, line 27 - page 11, line 11 *	1,3-7	C 07 K 17/00 C 12 M 3/00 A 61 K 35/14 C 12 N 5/08
X	JOURNAL OF IMMUNOLOGY vol. 143, no. 5, September 1989, Baltimore, USA pages 1461 - 1466; M. KOLBER et al.: "Target cell lysis by cytotoxic T lymphocytes redirected by antibody-coated polystyrene beads. " * the whole document *	1,3-7	
Y	EP-A-0 147 689 (ASAHI KASEI KOGYO KABUSHIKI KAISHA) * claims *	1-8	
Y	DE-A-3 634 017 (MAX PLANCK GESELLSCHAFT ZUR FÖRDERUNG DER WISSENSCHAFTEN E.V.) * the whole document *	1-8	
P,X	WO-A-9 004 633 (BRIGHAM AND WOMEN'S HOSPITAL) * claims *	1-8	

TECHNICAL FIELDS SEARCHED (Int. Cl.5)

C 07 K
C 12 M
A 61 K
C 12 N

The present search report has been drawn up for all claims

Place of search	Date of completion of search	Examiner
The Hague	28 November 90	NOOIJ F.J.M.

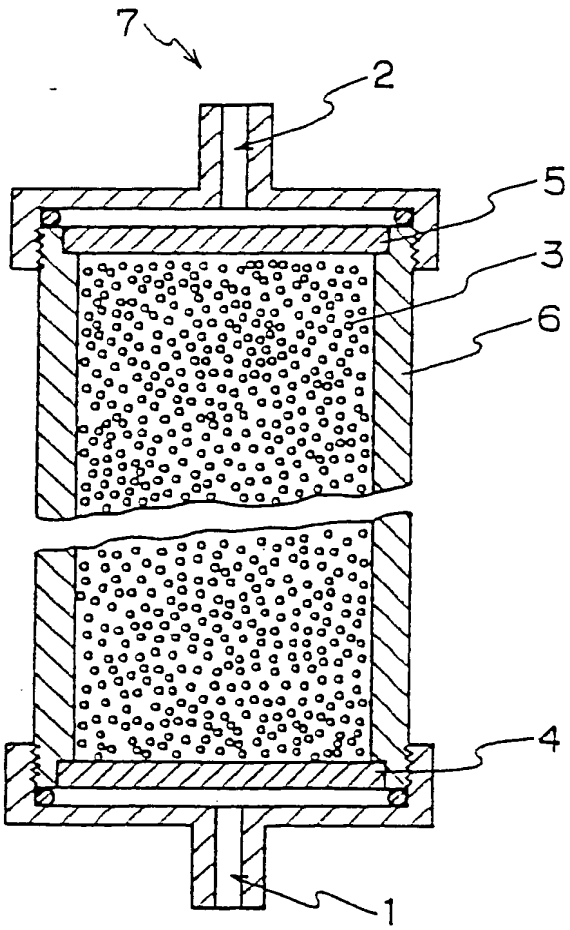
CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same catagory
A : technological background
O : non-written disclosure
P : intermediate document
T : theory or principle underlying the invention

E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

FIG.1



inducer can induce the tumor-lysing cells was estimated by the measurement of killer activity. As shown in Table 1 (the results of killer activity), the leukocytes conducted to the inducing operation could show higher killer activity than the leukocytes which were not conducted to the inducing opeation (control).

In order to make clear the effects obtained by fixing the anti-leukocyte antibodies, as to the case wherein the soluble antibodies (antibodies which were not fixed) were given in the medium and the stimulation was conducted for 24 hours (Comparative Example 1), the case wherein the same antibodies as used in Comparative Example 1 were fixed in the same incubator as used in Comparative Example 1 (96 wells microplate), and the stimulation was conducted for 20 hours (further the culture was conducted for 4 hours) (Example 2), and the case wherein the antibodies were fixed in the incubator, and the stimulating time was remarkably shortened (the stimulation was conducted for 10 minutes and the culture was conducted for 24 hours) (Example 3), the experimental results were shown in Tables 2, 3 and 6. From the results of the killer activity, it would be understood that in case of using the antibodies which were not fixed, the killer activity was low, on the other hand, in case of using the fixed antibodies, even if the stimulating time was made remarkably short, the killer activity was high, in other words, the effect for inducing tumor-lysing cells was high. This fact suggests that the change of morphology and shape can be prevented.

Further, in case of using the lection (PWM) as the stimulant, which was fixed, and inducing the tumor-lysing cells in the same manner as mentioned above, the inducing effect of tumor-lysing cells obtained in the case wherein the stimulation was conducted for 10 minutes (Comparative Example 3) was remarkably decreased compared to the effect obtained in the case wherein the stimulation was conducted for 20 hours (Comparative Example 2). On the other hand, as mentioned above, the killer activity could be kept high even if the stimulating time was shortened when fixing the anti-leukocyte antibodies (Example 3).

As apparent from the results of Examples 1-5 and Comparative Examples 1-3, when only adding the anti-leukocyte antibodies to the medium, the effect for inducing tumor-lysing cells (the killer activity) is slight. When the antibodies are fixed, the killer activity can be made so strong that anyone cannot expect by using even a small amount of the antibodies. Further, when inducing the tumor-lysing cells by using the fixed anti-leukocyte antibodies, the tumor-lysing cells can be induced even by very short time of the stimilation. Also, when the lection (PWM) which has hitherto been known as the tumor-lysing cell inducer is fixed in the same manner as in the case of fixing the anti-leukocyte antibodies and the tumor-lysing cells were induced by using the thus fixed lection, a long time of stimulating time is required and the obtained killer activity is low compared to the case inducing the tumor-lysing cells by using the fixed anti-leukocyte antibodies.

In addition to the ingredients used in the Examples, other ingredients can be used in the Examples as set forth in the specification to obtain substantially the same results.

Claims

1. A tumor-lysing cell inducer comprising an anti-leukocyte antibody or a part thereof, said antibody or said part being fixed in a water-insoluble carrier.

2. The inducer of Claim 1, wherein not less than two kinds of said anti-leukocyte antibodies or their parts are fixed in said carrier.

3. The inducer of Claim 1, wherein said anti-leukocyte antibody or said part thereof is fixed in the surface of said carrier in an amount of 1×10^{-9} μmol/cm^2 to 1×10^{-2} μmol/cm^2.

4. The inducer of Claim 1, wherein said carrier is hydrophilic.

5. The inducer of Claim 1, wherein said anti-leukocyte antibody is an anti-lymphocyte antibody.

6. The inducer of Claim 5, wherein said anti-lymphocyte antibody is an antibody against at least one surface antigen of the lymphocyte selected from the group consisting of CD2, CD3, CD4, CD8 and CD16.

7. A method for inducing tumor-lysing cells which comprises contacting a body fluid containing leukocytes with a tumor-lysing cell inducer, said tumor-lysing cell inducer comprising an anti-leukocyte antibody or a part thereof, said antibody or said part being fixed in a water-insoluble carrier.

8. A device for inducing tumor-lysing cells which comprises a tumor-lysing cell inducer comprising an anti-leukocyte antibody or a part thereof, said antibody or said part being fixed in a water-insoluble carrier, and a container having an inlet and an outlet for body fluid and a preventing means of the outpouring of said inducer from the container, said inducer being packed in said container.

which the antibody was not fixed, and the culture was conducted for 24 hours.

Measuring method of inducing effect

Amounts of glucoses consumed and the killer activity were measured in the same manner as in Example 1.

The results are shown in Table 7.

Table 7

Amount of glucose consumed		9.8 ± 0.6 %
Killer activity (against HeLa-S3)	(control)	7.9 ± 6.2 %
		55.1 ± 5.8 %
	(control)	20.5 ± 0.3 %

Comparative Example 3

Preparation of a tumor-lysing cell inducer

A tumor-lysing cell inducer was prepared in the same manner as in Comparative Example 2.

Preparation of a leukocyte suspension

A leukocyte suspension was prepared in the same manner as in Example 1.

Inducing operation

Into the tumor-lysing cell inducer was pipetted 200 μℓ/well (8×10^5 cells/mℓ) of the leukocyte suspension, and the stimulation was conducted for 10 minutes. Then, it was transferred to a microplate in which the antibody was not fixed and the culture was conducted for 24 hours.

Measuring method of inducing effect

Amounts of glucoses consumed and the killer activity were measured in the same manner as in Example 1.

The results are shown in Table 8.

Table 8

Amount of glucose consumed		5.4 ± 1.0 %
Killer activity (against HeLa-S3)	(control)	7.9 ± 6.2 %
		32.6 ± 1.8 %
	(control)	20.5 ± 0.3 %

In Example 1, the leukocyte suspension was contacted with the tumor-lysing cell inducer wherein the anti-leukocyte antibodies were fixed in the water-insoluble carrier, and how much the tumor-lysing cell

Comparative Example 1

Preparation of a solution of antibodies

A solution of antibodies (OKT 3, 25 μg/mℓ) was prepared.

Preparation of a leukocyte suspension

A leukocute suspension was prepared in the same manner as in Example 1.

Inducing operation

Into a flat bottom 96 wells microplate were pipetted 20 μℓ of the solution of antibodies (total amount of antibodies: 0.5 μg/well) and 200 μℓ/well (8×10^5 cells/mℓ) of the leukocyte suspension, and the culture was conducted for 24 hours.

Measuring method of inducing effect

Amounts of glucoses consumed and the killer activity were measured in the same manner as in Example 1.

The results are shown in Table 6.

Table 6

Amount of glucose consumed		20.2 ± 2.5 %
	(control)	7.9 ± 6.2 %
Killer activity (against HeLa-S3)		23.6 ± 4.1 %
	(control)	10.7 ± 3.3 %

Comparative Example 2

Preparation of a tumor-lysing cell inducer

Into a flat bottom 96 wells microplate was pipetted a solution of lectin (PWM, 25 μg/mℓ, 20 μℓ), it was allowed to stand at 4° C for 24 hours, and was washed with 200 μg of a serum-free medium (RPMI 1640) two times.

Preparation of a leukocyte suspension

A leukocyte suspension was prepared in the same manner as in Example 1.

Inducing operation

Into the tumor-lysing cell inducer was pipetted 200 μℓ/well (8×10^5 cells/mℓ) of the leukocyte suspension, and the stimulation was conducted for 20 hours. Then, it was transferred td a microplate in

Preparation of a tumor-lysing cell inducer

Four grams of CNBr-activated Sepharose 6MB was swelled with a little amount of a 1mM aqueous solution of HCℓ for 15 minutes, then washed with the 1 mM aqueous solution of HCℓ (800 mℓ), further a coupling buffer (0.5 M NaCℓ, 0.1 M $NaHCO_3$, pH 8.3, 20 mℓ).

To a solution wherein 25 μg/mℓ of OKT 3 and μg/mℓ of OKT 4 were dissolved in the coupling buffer was added the washed gels with a suck dry, and the reaction was conducted at 4° C overnight.

After washing with the coupling buffer, a blocking buffer (0.2 M glycine, 0.5 M NaCℓ, 0.1 M $NaHCO_3$, pH 8.3, 16 mℓ) was added to the reaction mixture, and the reaction was conducted at room temperature for 2 hours.

The obtained reaction mixture was washed with two kinds of post-treatment buffers total six times, that is, it was washed with a post-treatment buffer (0.5 M NaCℓ, 0.1 M AcNa, pH 4.0) alternately with a post-treatment buffer (0.5 M NaCℓ, 0.1 M Tris, pH 8.0), each washing being conducted three times.

Thus, a tumor-lysing cell inducer having 12 x 10^{-3} mmol/cm^2 of the anti-leukocyte antibodies fixed in the carrier was obtained.

Preparation of a leukocyte suspension

A peripheral blood containing heparine was placed on a layer of Ficoll-Paque in the state of a layer, and leukokccytes were separated by specific density centrifugation method. The obtained leukocyte fraction was washed with an isotonic solution thoroughly, and a concentration of the fraction was adjusted with an isotonic solution having a pH of 7.2 to 4 x 10^6 cells/mℓ.

Inducing operation

In a vinyl chloride tube [ø3 (ID) x 85 mm] was packed 0.6 mℓ of the obtained tumor-lysing cell inducer with saline (Japanese Pharmacopoeia, commercially available from Otsuka Seiyaku Kabushiki Kaisha), the both ends of the tube were covered with meshes (measuring tube of aggregating property of platelet, commercially available from Igaku Shoin Kiki Kabushiki Kaisha). The rinse was conducted by passing the same buffer solution as used in the leukocyte suspension through the tube, then the leukocyte suspension was passed through the tube at 25° C at a rate of 0.1 ma/minute.

Measuring method of inducing effect

Amounts of glucoses consumed and the killer activity were measured in the same miner as in Example 1.

The results are shown in Table 5.

Table 5

Amount of glucose consumed		6.2 ± 3.2 %
	(control)	5.7 ± 3.8 %
Killer activity (against HeLa-S3)		58.7 ± 4.2 %
	(control)	3.7 ± 4.0 %

In order to show the difference of the killer activity of the leukocytes between the case of using the fixed antibodies and the case of using the antibodies which were not fixed, experimental results of killer activity of leukocytes which were cultured with the antibodies which were not fixed were shown in Comparative Example 1.

An antibody (IgG) was cut into $F(ab')_2$ according to a usual manner. That is, 2 mℓ of antibodies (OKT 3, commercially available from Ortho Diagnostic Systems Inc.) with a concentration of 25 μg/mℓ was dialyzed against a 0.1 M sodium acetate buffer solution (pH 4.5), to which 1.0 μg of pepsin (commercially available from Toyoboseki Kabushiki Kaisha) was added. The mixture was allowed to stand at 37° C for 8 hours, then a pH of the resulting mixture was adjusted with a 1 N aqueous solution of sodium hydroxide to 8.0. The resulting mixture was passed through a Sephadex G-150 (commercially available from Pharmacia LKB Biotechnology Inc.) column (0.5 x 15 cm), using a 0.1 M sodium borate buffer solution (pH 8.0) as a developing solvent to separate an $F(ab')_2$ fraction. The $F(ab')_2$ fraction was dialyzed against a 0.1 M sodium phosphate buffer solution (pH 6.0).

Preparation of a tumor-lysing cell inducer

Four grams of CNBr-activated Sepharose 6MB was swelled with a little amount of a 1 mM aqueous solution of HCℓ for 15 minutes, and it was washed with the aqueous solution of HC_2 (800 mℓ), further a coupling buffer (0.5 M NaC_2, 0.1 M $NaHCO_3$, pH 8.3, 20 mℓ).

To a solution wherein the prepared $F(ab')_2$ was dissolved in the coupling buffer was added the washed gels with a suck dry, and the reaction was conducted at 4° C overnight.

After washing with the coupling buffer, a blocking buffer (0.2 M glycine, 0.5 M NaCℓ, 0.1 M $NaHCO_3$, pH 8.3, 16 mℓ) was added to the reaction mixture, and the reaction was conducted at room temperature for 2 hours.

The obtained reaction mixture was washed with two kinds of post-treatment buffers total six times, that is, it was washed with a post-treatment buffer (0.5 M NaCℓ, 0.1 M AcNa, pH 4.0) alternately with a post-treatment buffer (0.5 M NaCℓ, 0.1 M Tris, pH 8.0), each washing being conducted three times. Thus, a tumor-lysing cell inducer wherein 5 x 106 μmol/cm² of $F(ab')_2$ which was a part of anti-leukocyte antibody was fixed in the carrier was obtained.

Preparation of a leukocyte suspension

A solution wherein leukocytes were suspended was prepared in the same manner as in Example 1.

Inducing operation

Into the tumor-lysing cell inducer was pipetted 200 μℓ/well (8×10^5 cells/mℓ) of the leukocyte suspension, and the stimulation was conducted for 10 minutes. Then, it was transferred to a microplate in which the antibody was not fixed and the culture was conducted for 24 hours.

Measuring method of inducing effect

Amounts of glucoses consumed and the killer activity were measured in the same manner as in Example 1.

The results are shown in Table 4.

Table 4

Amount of glucose consumed		5.5 ± 1.7 %
	(control)	7.9 ± 6.2 %
Killer activity (against HeLa-S3)		59.2 ± 4.5 %
	(control)	20.5 ± 0.3 %

Example 5

Example 1.

The results are shown in Table 2.

Table 2

Amount of glucose consumed		22.5 ± 4.5 %
Killer activity (against HeLa-S3)	(control)	7.9 ± 6.2 %
		61.6 ± 3.5 %
	(control)	20.5 ± 0.3 %

Example 3

Preparation of a tumor-lysing cell inducer

A tumor-lysing cell inducer was prepared in the same manner as in Example 2.

Preparation of a leukocyte suspension

A solution wherein leukocytes were floated was prepared in the same manner as in Example 1.

Inducing operation

Into the tumor-lysing cell inducer was pipetted 200 $\mu\ell$/well (8×10^5 cells/mℓ) of the leukocyte suspension and the stimulation was conducted for 10 minutes. Then, it was transferred to a microplate in which the antibody was not fixed and the culture was conducted for 24 hours.

Measuring method of inducing effect

Amounts of glucoses consumed and the killer activity were measured in the same manner as in Example 1.

The results are shown in Table 3.

Table 3

Amount of glucose consumed		3.2 ± 2.1 %
Killer activity (against HeLa-S3)	(control)	7.9 ± 6.2 %
		51.2 ± 2.2 %
	(control)	20.5 ± 0.3 %

Example 4

Preparation of a part of antibody, [$F(ab')_2$]

(1) Measurement of an amount of glucose consumed

After stimulating, a concentration of the leukocyte suspension was adjusted to 4×10^6 cells/mℓ, which was used as a sample. Also, a concentration of the leukocyte suspension which was not stimilated was adjusted to the same concentration as the sample, which was used as a control. The sample and the control were cultured respectively on a FCS 10 % RPMI 1640 medium in a carbon dioxide incubator for 24 hours. Glucose concentrations in the media before and after the culture were measured (glucose B test Wako, commercially available from Wako Purechemical Industries, Ltd.), and an amount of the glucose consumed was calculated.

(2) Measurement of killer activity

After stimulating, a concentration of the leukocyte suspension was adjusted to 4×10^6 cells/mℓ, which was used as a sample. Also, a concentration of the leukocyte suspension which was not stimulated was adjusted to the same concentration as the sample, which was used as a control. The sample and the control were cultured respectively on an RPMI 1640 medium in a carbon dioxide incubator for 24 hours. Killer activity against HeLa-S3 cells after culturing was measured according to ^{51}Cr-release method.

The result are shown in Table 1.

Table 1

Amount of glucose consumed		10.2 ± 2.4 %
Killer activity (against HeLa-S3)	(control)	5.7 ± 3.8 %
		43.7 ± 2.8 %
	(control)	3.7 ± 4.0 %

Example 2

Preparation of a tumor-lysing cell inducer

Into a flat bottom 96 wells microplate was pipetted a solution of antibodies (OKT 3, 25 μg/mℓ, 20 μℓ), then, it was allowed to stand at 4° C for 24 hours and was washed with 200 μℓ of a serum-free medium (RPMI 1640) two times to fix the antibodies. An amount of the fixed antibodies was measured by ELISA method to give a result of 0.38 μg/well (6.3×10^{-5} μmol/cm^2).

Preparation of a leukocyte suspension

A leukocyte suspension was prepared in the same manner as in Example 1.

Inducing operation

Into the tumor-lysing cell inducer was pipetted 200 μℓ/well (8×10^5 cells/mℓ) of the leukocyte suspension, and the culture was conducted for 20 hours while stimulating. It was transferred to a microplate in which the antibody was not fixed, and the culture was conducted for 4 hours.

Measuring method of inducing effect

Amounts of glucoses consumed and the killer activity were measured in the same manner as in

Next, the device for inducing tumor-lysing cells of the present invention, utilizing the inducer as mentioned above is explained by means of Fig. 1 which is a cross sectional view of one embodiment of the devices for inducing tumor-lysing cells of the present invention.

In Fig. 1, 1 shows an inlet of a body fluid, 2 shows an outlet of the body fluid, 3 shows the tumor-lysing cell inducer of the present invention, 4 and 5 show filters through which the body fluid and ingredients contained in the body fluid can be passed but the tumor-lysing cell inducer cannot be passed, 6 shows a column, and 7 shows a container.

A shape and a kind of a material of the container 7 are not particularly limited. Preferable examples of the containers are, for instance cylindrical containers having a volume of about 150 to 400 mℓ and a diameter of about 4 to 10 cm, and the like.

The present invention is more specifically described and explained by means of the following Examples in which all percents and parts are by weight unless otherwise noted. It is to be understood that the present invention is not limited to the Examples and various changes and modifications may be made in the invention without departing from the spirit and scope thereof.

Example 1

Preparation of a tumor-lysing cell inducer

Four grams of CNBr-activated Sepharose 6MB (which was commercially available from Pharmacia LKB Biotechnology Inc.) was swelled with a little amount of a 1mM aqueous solution of HCℓ for 15 minutes, then washed with 800 mℓ of the 1mM aqueous solution of HCℓ, then a coupling buffer (0.5 M NaCℓ, 0.1 M $NaHCO_3$, pH 8.3, 20 mℓ).

To a solution wherein 25 μg/ml of antibodies (OKT 3 which was commercially available from Ortho Diagnostic Systems Inc.) was dissolved in the coupling buffer was added the washed gels with a suck dry, and the mixture was reacted at 4 °C overnight.

After washing with the coupling buffer, a blocking buffer (0.2 M glycine, 0.5 M NaCℓ, 0.1 M $NaHCO_3$, pH 8.3, 16 mℓ) was added to the reaction mixture, and the reaction was conducted at room temperature for 2 hours.

The obtained reaction mixture was washed with two kinds of post-treatment buffers total six times, that is, it was washed with a post-treatment buffer (0.5 M NaCℓ, 0.1 M AcNa, pH 4.0) alternately with a post-treatment buffer (0.5 M NaCℓ, 0.1 M Tris, pH 8.0), each washing being conducted three times.

Preparation of a solution wherein leukocytes were suspended (leukocyte suspension)

A peripheral blood containing heparine was placed on a layer of Ficoll-Paque (commercially available from Pharmacia LBK Biotechnology Inc.) in the state of a layer, the leukocytes were separated by specific density centrifugation method. The obtained leukocyte fraction was washed with an isotonic solution thoroughly, and a concentration of the washed fraction was adjusted with an isotonic solution having a pH of 7.2 to 4×10^6 cells/mℓ.

Inducing operation

In a vinyl chloride tube [ø 3 (ID) x 85 mm] was packed 0.6 mℓ of the obtained tumor-lysing cell inducer with saline (Japanese Pharmacopoeia, commercially available from Otsuka Seiyaku Kabushiki Kaisha), and the both ends of the tube were covered with meshes (measuring tubes of aggregating property of platelet, commercially available from Igaku Shoin Kiki Kabushiki Kaisha). The rinse was conducted by passing the same buffer solution as used in the leukocyte suspension through the tube, then the leukocyte suspension was passed through the tube at 25 °C at a rate of 0.1 mℓ/minute.

Measuring method of inducing effect

exerting on the antibody molecule as it is, the antibody is binded with the carrier through an ester (including a thioester) bond, an amido (including a sulfonamido) bond, an ether (including a thioether) bond, amino bond, imide bond, and the like. The bonds are not limited thereto. Also, there is a way wherein after introducing a functional group having high reactivity into the antibody molecule, the antibody is binded with the water-insoluble carrier. There are various agents used for introducing functional groups having higher reactivity into the antibody, and DSC and WSC are particularly effective as the agent for introducing sfunctional groups.

It is preferable that the tumor-lysing cell inducer of the present invention hash proper amount of the anti-leukocyte antibody on the carrier. When the amount of the anti-leukocyte antibody is small to excess, the inducer has little the effect by the anti-leukocyte antibody. On the other hand, when the amount is large to excess, the nonspecific adsorption and cytotoxicity are caused. That is, it is preferable that the tumor-lysing cell inducer has from 1×10^{-9} μmol/cm² to 1×10^{-2} μmol/cm², more preferably from 5×10^{-6} μmol/cm² to 5×10^{-4} μmol/cm², of the fixed anti-leukocyte antibody on its surface.

The tumor-lysing cell inducer of the invention is one wherein the anti-leukocyte antibody or the part thereof is fixed in the water-insoluble carrier and can have any shape, for instance it is in the shape of particles, a plate, a membrane, a fiber and the like. When the tumor-lysing cell inducer packed in a column is used, there is preferable an inducer capable of forming gaps through which cells contained in a body fluid can sufficiently pass. For instance, when the inducer is in the shape of the particles, the powdery inducer is not preferable and particles having a particle size of not less than 200 μm are preferable. More detailedly, it is preferable as the inducer to use particles from which particles having a particle size being small to excess and particles having a particle size being large to excess are removed. Further, it is more preferable to use particles having an average particle size of 200 to 1,000 μm and having a narrow particle size distribution. Also, when the inducer is in the shape of a hollow fiber, it is preferable that an inside diameter of the hollow fiber is not less than 5 μm. When the inducer is in the shape of a fiber which is not hollow, it is preferable that an outside diameter is not less than 1 μm.

It is preferable that the surface of the tumor-lysing cell inducer is smooth. When the surface is rough, the nonspecific adsorption is increased and the selective activation is lowered.

The thus obtained tumor-lysing cell inducer by fixing the anti-leukocyte antibody or its part in the solid, the water-insoluble carrier can produce the excellent effect for activating leukocytes, which cannot be expected from soluble antibodies.

The term "tumor-lysing cell" used herein means a cell which is derived from a leukocyte and has a function capable of lysing tumor cells.

In general, a case that cells are stimulated to induce a certain function from the cells is called "the activation of cells", and there are various kinds of the activations. Typical examples of the activations (and its measuring method) are described as below.

(1) The proliferation faster accelerates than usual. (an amount of uptake of 3H-TdR, MTT method, a consumed amount of glucose)

(2) A produced amount of IL-2 is increased. (method according to IL-2-dependent cells)

(3) An amount of IL-2 receptor is increased. (a labeled antibody method using IL-2-dependent cells)

(4) Killer activation (cytotoxicity property against other cells is measured)

The term "tumor-lysing cell induction" used herein means the activation concerned to the killer activation as mentioned above.

Any way can apply to the method for inducing the tumor-lysing cells from the leukocyte in the body fluid, using the tumor-lysing cell inducer of the present invention.

Typical examples of the inducing methods are, for instance, a method in which a body fluid removed from a body is packed in a bag, the tumor-lysing cell inducer is mixed with the body fluid in the bag to induce the tumor-lysing cells, and the tumor-lysing cell inducer is filtered off to obtain the body fluid containing the tumor-lysing cells; a column method in which a body fluid is passed through the inducers packed in a container having an inlet and an outlet for body fluid, and a filter through which the body fluid can be passed but the tumor-lysing cell inducer is not passed, the filter being attached to the outlet; and the like. Both methods can be used. The column method is easy in operation. Further, according to the column method, tumor-lysing cells can be obtained from a body fluid, particularly leukocytes in a blood, of a patient by incorporating the column wherein the inducer is packed into an extracorporeal circulation. The tumor-lysing cell inducers of the present invention are suitable for use of the column method.

There is preferable a method wherein the tumor-lysing cell inducers are packed in the column, the column wherein the inducers are packed is incorporated into an extracorporeal circulation, and tumor-lysing cells are induced according to the on-line information processing system, since according to such a method, a large amount of the body fluid can be treated in a short time.

anti-leukocyte antibody or a part thereof, the antibody or the part being fixed in a water-insoluble carrier.

Also, in accordance with the present invention, there is provided a method for inducing tumor-lysing cells which is comprises contacting a body fluid containing leukocytes with the inducer as mentioned above.

Further, in accordance with the present invention, there is provided a device for inducing tumor-lysing cells which comprises the inducer as mentioned above and a container having an inlet and an outlet for body fluid and a preventing means of the outpouring of the inducer from the container, the inducer being packed in the container.

The tumor-lysing cell inducer of the present invention is safe and has strong killer activity compared to any stimulants capable of inducing tumor-lysing cells which have hitherto been known.

Fig. 1 is a cross sectional view of one embodiment of the devices for inducing tumor-lysing cells of the present invention.

The term "body fluid" used herein means a humoral liquid component derived from a living body, for instance, blood, plasma, serum, ascites, lymph liquid, synovia in articular cavity, fractions obtained therefrom, and the like.

The term "anti-leukocyte antibody" used herein means an antibody prepared by using a leukocyte as an antigen. The leukocytes include a lymphocyte, a monocyte, a neutrophil, a basophil, an eosinophil, and the like. Among anti-leukocyte antibodies, an anti-lymphocyte antibody is preferred. The lymphocyte has various surface antigens on its surface. It has been decided that the surface antigens are called by a CD code (cluster differentiation) in an international workshop. Among the antibodies against the surface antigens of the lymphocyte, antibodies against CD2, CD3, CD4, CD8 and CD16 are particularly preferred. In the selection of an antibody to be fixed from among the anti-leukocyte antibodies, various kinds of antibodies against various kinds of antigens may be used as well as one kind of an antibody against one kind of an antigen may be used. In case of using one kind of the antibody, the antibody against CD2, CD3 or CD16 is particularly preferable, though it is not limited thereto. Also, in case of using various kinds of the antibodies, mixtures containing the antibody against CD2, CD3 or CD16 are particularly preferable, though various kinds of the mixtures are considered.

As the kinds of the antibody itself, there are IgG, IgM, IgA, IgD, IgE, and the like, and any kinds of antibodies can be used. Also, there are antibodies derived from animals such as a mouse, a rat, a rabbit and a goat and antibodies derived from a human. The antibodies derived from humans are preferable from the viewpoint of the safety including the antigenicity, though there is no particular limitation as to the antibody.

The term "a part of the antibody" used herein means a fragment of the antibody or a plurality of the fragments combined with covalent bond and having a lower molecular weight than the molecular weight of the antibody. The antibody can be severed in any method, for instance, it is served by using an enzyme or a chemical agent, or is genetically engineeringly severed.

The water-insoluble carrier used in the present invention is in the state of a solid at the ordinal temperature under original pressure and is water-insoluble. It is desirable that the carrier has at least one functional group on its surface, for binding the anti-leukocyte antibody or the part thereof with the carrier.

Examples of the carrier, for instance, an inorganic carrier such as glass beads or a silica gel; an organic carrier such as a synthetic polymer e.g. crosslinked polyvinyl alcohol, crosslinked polyacrylate, crosslinked polyacrylamide or crosslinked polystyrene, or a polysaccharide e.g. crystalline cellulose, crosslinked cellulose, crosslinked agarose or crosslinked dextran; a complex carrier such as a carrier made of the two kinds of the organic carrier, a carrier made of the organic carrier and the inorganic carrier.

Examples of the functional groups existing on the carrier surface are, for instance, hydroxyl group, amino group, aldehyde group, carboxyl group, thiol group, silanol group, amido group, epoxy group, a halogen atom, succinylimide group, an acid anhydride group, and the like. The groups are not limited thereto.

Also, it is preferable that the water-insoluble carrier is hydrophilic, for preventing the deterioration of the tumor-lysing cells, which is caused by adhering the cells to the surface of the tumor-lysing cell inducer with nonspecifically hydrophobic interaction.

The tumor-lysing cell inducers of the present invention are characterized in that the anti-leukocyte antibody or the part thereof is fixed in the water-insoluble carrier. For obtaining such tumor-lysing cell inducers, the anti-leukocyte antibody can be fixed in the water-insoluble carrier in any way, for instance with covalent bond, hydrophobic bond or ion bond. Among them, it is particularly preferable that the antibody is fixed in the carrier by covalent bond from the viewpoint of the safety during the sterilization or treatment.

In case of fixing the antibody in the water-insoluble carrier with covalent bond, there are various ways and any ways can be applied to the present invention. For instance, when utilizing a functional group

TUMOR-LYSING CELL INDUCER, METHOD FOR INDUCING TUMOR-LYSING CELL AND DEVICE FOR INDUCING TUMOR-LYSING CELL

The present invention relates to a tumor-lysing cell inducer which induces cells capable of lysing tumors by the stimulation of leukocytes in a body fluid, and a method for inducing tumor-lysing cells and an inducing device of tumor-lysing cells, which utilize the above-mentioned inducer.

It has been known that in leukocyte fractions, there are cells which have a function for lysing or excluding tumor cells, such as a cytotoxic T lymphocyte, a natural killer cell and a monocyte. Not only healthy persons but also tumor-bearing patients have these cells in their bodies. Nevertheless, the tumor in the tumor-bearing patient grows, thus his symptom becomes worse. It has been considered that such a phenomenon is caused by the decrease of the number of the tumor-lysing cells and the lowering of the activity of the tumor-lysing cells due to the immunological tolerance, the activation of immunosuppresive cells, the shift toward immunosuppressive side in humoral immune factor balance, and the like.

As one of treatments of malignant tumors, it can be considered that tumor-lysing cells are obtained by stimulating leukocytes. It is very difficult, however, that the tumor-lysing cells are induced by stimulating efficiently and selectively the leukocytes in vivo . Accordingly, a treatment of tumors wherein leukocytes are taken out from a tumor-bearing patient's body, the leukocytes are cultured with stimulating for few days in vitro while adjusting artificially the environment to induce tumor-lysing cells, and the induced tumor-lysing cells are returned into the tumor-bearing patient's body is tried in various ways. As stimulants used in the above-mentioned methods, there have been known lymphokines such as interleukin 2 [LAK treatment: E. A. Grimm, Journal of Experimental Medicine, 155 , 1823 (1982)], lectins such as PHA and PWM, protein A, and the like.

Presently, for treatment of malignant tumors, trials wherein lymphocytes are cultured with stimulating in vitro to induce tumor-lysing cello have been lively studied. The treatments have, however, some problems such that (1) a stimulant used in the induction of the tumor-lysing cells have high toxicity, so there is a great risk if leaking the stimulant; (2) the tumor-lysing cells are changed in morphology and shape during the few days' culture; and (3) troublesome operations with the culture are required and the contamination is caused.

In order to solve the problem (1), it is considered that a stimulant having low toxicity is used and the stimulant is fixed in an insoluble carrier to prevent the leakage of stimulant. As to the stimulant having low toxicity, antibodies against leukocytes are laboratorially studied in part. It has been generally known, however, that substances having low toxicity are low in effect for inducing tumor-lysing cells. Therefore, an effect for inducing tumor-lysing cells by leukocyte antibodies is not very high, for instance, it is only reported to induce tumor-lysing cells having antigenic nonspecificity from tumor-lysing cells having antigenic specificity [J. F. M. Leeuwemberg, Journal of Immunology, 134 , (6), 3770(1985)]. Further, since it is general that there are many cases where the bioactivity is lowered by fixing the stimulant, it can be assumed that when the leukocyte antibody is fixed, it is not expected to obtain an excellent effect for inducing tumor-lysing cells.

Also, in order to solve the problems (2) and (3), the shortening of the duration of culture or the omission of culture itself is required. It is essential, anyway, to strongly stimulate the leukocytes. Stimulants having strong inducing activity of tumor-lysing cells are, as mentioned above, generally high in toxicity, and even if using the stimulant fixed in an insoluble carrier, it is very difficult to completely prevent the leakage of the stimulant. That is to say, it is almost impossible to put a safe and effective stimulant into a practical use from the view point of safety.

In order to develop a tumor-lysing cell inducer having the safety as well as the high activity for inducing tumor-lysing cells, it is required to obtain simultaneously two properties contradictory to each other as mentioned above, which is very difficult, and, in fact, such an inducer has not yet been realized.

An object of the present invention is to provide a safe and highly effective tumor-lysing cell inducer for treatment of tumors.

A Further object of the present invention is to provide a method for inducing tumor-lysing cells using the inducer as mentioned above.

A still further object of the present invention is to provide a device inducing tumor-lysing cells utilizing the inducer as mentioned above.

These and other objects of the present invention will become apparent from the description hereinafter.

It has now been found that when an anti-leukocyte antibody or a part thereof fixed in a solid-phase is used, tumor-lysing cells having high activity which cannot be not expected from the antibody which is not fixed can be induced.

In accordance with the present invention, there is provided a tumor-lysing cell inducer comprising an

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(71) Applicant: KANEGAFUCHI KAGAKU KOGYO KABUSHIKI KAISHA
2-4 Nakanoshima 3-chome
Kita-ku Osaka-shi Osaka-fu(JP)

(72) Inventor: Eiji, Ogino
1-7, Oishihigashi-machi 4-chome
Nada-ku, Kobe-shi, Hyogo-ken(JP)
Inventor: Nobutaka, Tani
17-29, Fuminosato 4-chome
Abeno-ku, Osaka-shi, Osaka-fu(JP)
Inventor: Toshio, Hayami
21-23, Sakamoto, Noguchi-cho
Kakogawa-shi, Hyogo-ken(JP)

(74) Representative: Türk, Gille, Hrabal
Brucknerstrasse 20
W-4000 Düsseldorf 13(DE)

(54) **Tumor-lysing cell inducer, method for inducing tumor-lysing cell and device for inducing tumor-lysing cell.**

(57) A tumor-lysing cell inducer comprising an anti-leukocyte antibody or a part thereof, the antibody or the part being fixed in a water-insoluble carrier, a method for inducing tumor-lysing cells and an inducing device of tumor-lysing cells, utilizing the inducer as mentioned above. When inducing the tumor-lysing cells by using the inducer of the present invention, the obtained killer activity is so strong that anyone cannot expect and furthermore such an activity can be obtained by stimulating with a small amount of the inducer in a short time.

FIG.1